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MYCOTOXINS IN ANIMAL FEEDS: The Extent and Nature of the Problem

Aflatoxin presents a problem in the Southeast, where a high percentage of the corn may be contaminated. There are sporadic problems in the Midwest, but more survey data are needed from that region.

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The title of this manuscript may perhaps be better phrased as a question: What is the extent and nature of the mycotoxin problem in the animal industry? This question is difficult to answer because we have not as yet definitively defined the extent of mycotoxin contamination of feed components. In fact, this definition is still the goal of much of the mycotoxin work in progress.

Perhaps more disturbing is the possibility that the question will have to be asked on a continuing basis, i.e. all feeds may have to be constantly monitored for the presence of mycotoxins to ensure their safety to consuming animals. I believe the rationale behind this point is obvious when you realize that grains and other feed components can become contaminated in any phase of the production cycle starting with potential mold growth in the field and continuing on through harvest, storage, processing, shipment and even during the period when the feed is placed out for the animals to consume.

Modern technology may also contribute to the problem. Harvesting corn, for example, is carried out at increasingly higher moisture levels by picker-shellers that damage many kernels. This damage leaves the kernels with increased susceptibility to mold invasion.

Another complicating factor is the difficulty in extrapolating to field conditions toxicity data developed in the laboratory. This aspect has not received the research attention it deserves because the scientist in the laboratory prefers to minimize the variables he has to deal with.

In the field, the picture may be much more complex. There is evidence to indicate that the degree of toxicity of a given mycotoxin is affected by a host of interrelating factors — e.g. the breed, sex, age and nutritional state of the host, the environment, the presence of multiple toxins, synergistic effects accruing from the presence of nontoxic substances, binding of toxin to substrate molecules as well as to host molecules, and conversion of the toxin by the host to substances having more or less toxicity.

Even the concept of toxicity needs some definition. Although acute toxicity is dramatic and can result in sharp losses at any given time — e.g. I visited one farm where $70,000 worth of turkeys had died from aflatoxicosis and another $30,000 worth were of dubious market value — I believe that subacute doses which result in lowered feed efficiency and poorer weight gains may be of over-all greater economic impact.

Such effects are insidious, hence difficult to detect. Carcinogenicity of certain mycotoxins, while of considerable import to humans, probably has little economic impact on the farm — the relatively short time span required to bring a farm animal or bird to market weight probably would preclude worry about carcinogenic effects. The only exception I am aware of could be hatchery-raised rainbow trout that are usually susceptible to aflatoxin-induced hepatomas.

Important Toxins

What toxins should the feed industry be concerned with? Two or three immediately come to mind that are undoubtedly of importance. Obviously, the first is the aflatoxins, and the second represents a group of toxins produced by the Fusaria that can be subdivided into zearalenone, an estrogenic metabolite, and the 12,13-epoxy-trichothecenes. In this country, there is less certainty about the nephrotoxins (ochratoxin and citrinin) the tremorgenic and the Alternaria toxins, patulin and penicillic acid.

The aflatoxins constitute a family of 14 naturally-occurring toxins. Of these, aflatoxin B_1 is of primary concern (Figure 1) since it is the most toxic and occurs most often. A brief review of the dietary aflatoxin concentrations causing toxicosis is shown in Table 1.

As to the extent of occurrence in feed components in this country, primary concern rests with corn and with cottonseed meal, the latter being used principally in trout rations. Extensive surveys at the Northern Laboratory indicate that aflatoxin contamination is not a particular problem in the midwest Corn Belt. Corn from the crop years 1964, 1965, 1967 and 1968-69 indicated an incidence of only 2.5 to 2.7%, mostly in sample grade and with a range of only 3 to 37 ppb (Shotwell et al., 1969). However, a survey in the 1969-1970 period revealed that in the Southeast, 35% of 60 samples assayed were positive for aflatoxin, the contamination being found in all grades and with a range of 6 to 348 ppb (Shotwell et al., 1973).

Since then, Lillehoj and his associates at the Northern Laboratory have examined 1973 crop corn directly at harvest in the Southeast for the presence of aflatoxin (Lillehoj et al., in press). Their results add a new dimension to the problem. It had been assumed that aflatoxin contamination resulted primarily from inadequate grain storage. Of the 297 samples they collected, either from field sites or at the elevator delivery point, 152 or 51% were positive for aflatoxin, and of these, 94 or 31.6% had 20 ppb or higher. The levels ranged from less than 9 to more than 640 ppb.

If the observations made in this latest survey are extrapolated to the 500 million bushels of corn grown in the 15 southern states in 1973, about 160 million bushels of that crop could be expected to contain at least 20 ppb B_1, and 50 million bushels would be contaminated with levels exceeding 100 ppb. The implications are obvious. Before long-range research can be brought to bear to solve this problem, it may become necessary to detoxify a good share of this contaminated corn. One approach utilizing ammonia is under study at the Northern Laboratory, and we hope soon to begin, under the direction of the Richard Russell Research Center, the long-term animal studies necessary to obtain FDA clearance.

TABLE 1. Dietary Aflatoxin Concentrations Causing Toxicosis

Species	Age	Aflatoxin Content (ppm)	Duration of Feeding	Effects
Calves	Weanling	0.2-2.2	16 weeks	Stunting, death, liver damage
Steers	2 years	0.2-0.7	20 weeks	Liver damage
Cows	2 years	2.4	7 months	Liver damage
Pigs	Newborn	0.23	4 days	Stunting
Pigs	2 weeks	0.17	23 days	Anorexia, stunting, jaundice
Pigs	4-6 weeks	0.4-0.7	3-6 months	Stunting, liver damage
Chickens	1+ week	0.8	10 weeks	Stunting, liver damage
Ducks	Unknown	0.3	6 weeks	Liver damage, death

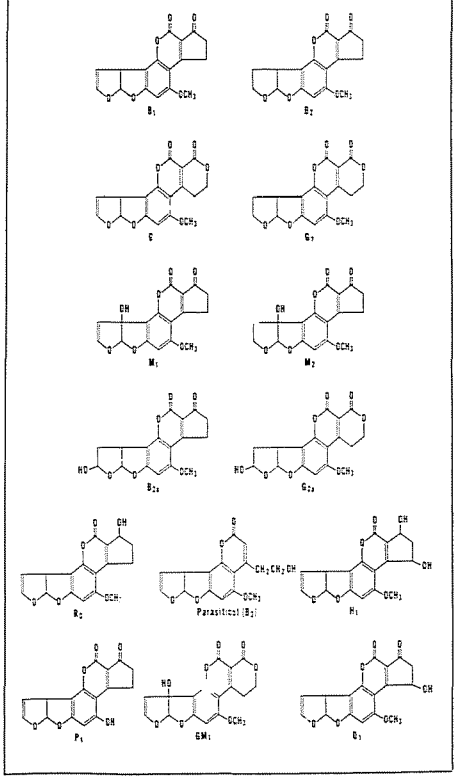
EDITOR'S NOTE: This is the third in a series of papers presented at the 1975 Animal Nutrition Research Council meeting in Washington.

Cottonseed Meal

Cottonseed meal is also a problem with respect to aflatoxin contamination, for, again, toxin formation appears to originate in the field. A three-year survey (1964-1967) revealed an 8% incidence of detectable aflatoxin in cottonseed and a 19% incidence in meal derived from that seed; average aflatoxin levels were 143 and 99 ug/kg respectively (Whitten, 1968). Our sister laboratory in New Orleans, the Southern Regional Research Center, has developed a detoxification process based on the use of ammonia under pressure; here again, FDA clearance is being sought (Mann et al., 1971).

The *Fusarium*-produced toxins present a somewhat different problem, one in which research data are even more meager. Fusarial contamination of grains appears to be associated with cool, wet weather. Tuite et al. (1974) at Purdue University, Lafayette, Ind., carried out an extensive analysis of the weather and the occurrence of *Fusarium* ear rot

FIGURE 1. Structures of naturally occurring afflatoxins.



epidemics of corn in Indiana in 1965 and in 1972.

Ideal conditions for an outbreak appeared to be about nine days of rain and a mean temperature below 70° F (21° C) during silking. Crop yields are reduced, but more importantly, there is considerable loss in quality and acceptability of contaminated grain as a feed. The problem is compounded because *Fusaria* are capable of producing a number of toxins, some of which have not as yet been identified or had their importance ascertained, nor have assays been developed for all the known toxins. This lack has hampered carrying out the surveys necessary to determine the extent of the whole problem.

The two *Fusarium* toxins of most concern to the feed industry are zearalenone (Figure 2) and the trichothecenes (Figure 3). Shotwell et al. (1971) assayed 293 corn samples from 1968 to 1969 and found zearalenone in only five samples at levels ranging from 450 to 750 ppb. In 1972, when the midwest Corn Belt was hit hard by *Fusarium* infection, an assay of 223 corn samples by FDA (Eppley et al., 1974) showed zearalenone to be present in 17% of the samples and a rabbit skin-irritant to be present in 93 of 173 samples (53.7%).

The literature on zearalenone is particularly exasperating; although this toxin has been involved in stillbirths and estrogenic effects in swine (Miller et al., 1973; Mirocha et al., 1967) and in cattle (Mirocha et al., 1968; Roine et al., 1971), quantitative data on toxicity are not available and toxicological analyses are sparse. Nevertheless, it is fairly certain that zearalenone plays a role in mycotoxicoses, but the extent remains to be defined although Stoloff *et al.* (1975) reported that 6% of 315 samples collected in the Corn Belt contained this toxin.

Of equal interest has been the problem associated with *Fusarium*-contaminated grain (scabby grain) which often results in swine refusal to eat or vomiting upon consumption of small quantities. This problem has been documented since 1914 throughout the world, but reached particularly severe levels in 1972 in the Midwest, when conditions were conducive for corn contamination by *Fusarium* species, *F. graminearum* in particular.

It is difficult to estimate a monetary loss associated with such corn, except to assume that it was considerable. By December 1973, Vesonder et al. (1973) at the Northern Laboratory had isolated the toxin directly from contaminated corn and showed it to be a new trichothecene to which the picturesque name, vomitoxin, was assigned. However, this isolation still left the problem of the factor causing refusal. We have now resolved the question and have found that the refusal factor is identical to the vomition factor. The literature is quite confusing on this subject because a number of trichothecenes can cause vomiting (Figure 3). However, none of these compounds, other than vomitoxin, have actually been isolated from a naturally-contaminated grain and identified as the causative agent responsible for refusal to eat or vomiting in swine, the principal animal affected in the U.S.

Trichothecenes

Trichothecenes are extremely stable compounds, and it does not appear at the moment that an economical or technically feasible detoxification process can be developed. Attempts to mask the unpalatability of contaminated corn have met with failure. Blending contaminated corn with good corn may result in consumption by swine, but feed efficiency and weight gains still will be reduced.

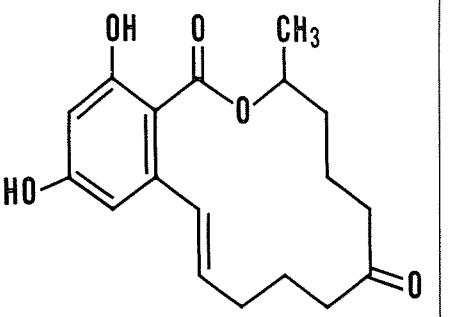
A number of other mycotoxins have been isolated, and in some cases there is circumstantial evidence for their involvement in mycotoxicoses. A good example would be the tremorgenic toxins produced by a variety of Penicillia that have been implicated in mycotoxicoses in sheep, horses and cows (Wilson et al., 1968; Ciegler, 1969). Although Bermuda grass toxicosis also seems to involve a tremorgen, no definitive information is available.

The nephrotoxins (ochratoxin and citrinin) have been implicated in swine mycotoxicoses in Denmark and, possibly, a human nephrosis in the Balkans, but their effect, if any, in the U.S. is not known.

The two lactones, patulin and penicillic acid, have been found occurring naturally in the United States, but their importance to the feed industry has not yet been determined. We have shown that penicillic acid is readily produced in considerable quantities by fungi responsible for "blue-eye" disease of corn, but definitive surveys in the field for this toxin have not been undertaken.

In summary, aflatoxin appears to present a problem to the feed industry primarily in southeastern states, where a high percentage of the corn may be contaminated. Zearalenone and the trichothecenes cause sporadic problems in the Midwest, but extensive survey data are not available to delineate the situation more accurately. Sufficient research has not been conducted on other mycotoxins to permit a definitive statement.

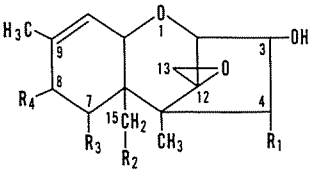
FIGURE 2. Structure of F-2 (zearalenone).



$C_{18}O_5H_{22}$

Structure of F-2 (Zearalenone)

FIGURE 3. Structures of emetic trichothecenes.



	R_1	R_2	R_3	R_4
Nivalenol	OH	OH	OH	=O
Fusarenon-X	OAc	OH	OH	=O
T-2	OAc	OAc	H_2	$OCOCH_2CH(CH_3)_2$
Vomitoxin	H_2	OH	OH	=O

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